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LUMINESCENCE CONTROL IN THE TUBE-WORM *CHAETOPTERUS VARIOPEDATUS*: ROLE OF NERVE CORD AND PHOTOGENIC GLAND

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Abstract

Electrical and mechanical stimulation of the parapodial epidermis of *Chaetopterus variopedatus* evoked luminescence which was propagated only slightly to adjacent ipsilateral, but not to contralateral, parapods. In contrast, electrical stimulation of the highly modified aliform notopods led to propagation of luminescence through the entire body. Above a critical stimulus threshold, stimulation of the ventral nerve cord at any level evoked luminescence which was through-conducted. Only stimulation of the cerebral ganglia could bring about an orderly antero-posterior sequence of luminescence propagation. Discharges of nerve cord impulses invariably preceded the onset of spontaneous or electrically stimulated luminescence, and the propagation of both activities was interrupted by section of the nerve cord. Mechanical stimulation of parapods also evoked impulses at the corresponding level in the nerve cord. A large photogenic gland lying on the dorso-median surface of the 10–12th segments was refractory to electrical and mild mechanical stimulation, but responded by releasing large amounts of luminescent mucus after rupture of its epithelium. Mechanical agitation of the tube was quickly followed by the ejection of a cloud of luminescent mucus through one end, and readjustment of the worm's position to the other end of the tube. Epithelial luminescent activities are coordinated by the ventral nerve cord and luminescent discharges from the photogenic gland appear to be associated with defensive and tube cleaning activities.

Introduction

The bioluminescence of *Chaetopterus variopedatus* has puzzled naturalists for more than a century because little use could be made of light emitted by an animal always confined inside its tube, which is itself largely buried in sandy bottoms (Enders, 1909; Dahlgren, 1916; Harvey, 1952). Nicol (1962) postulated that the extracellular luminescent mucus produced by the worm's epithelium could serve to repel photonegative intruders, such as the small crustaceans often found inside the tubes (pers. obs.). These speculations have proved difficult to test experimentally or by field observations because of the secretive habits of this worm.

An alternative and more tractable approach is to make observations in the laboratory of behavioral activities accompanying light emission and to investigate the role of the nervous system in the coordination of luminescence. Previous investigators have focused on the effects of various stimuli on luminescence of "intact" worms (Nicol, 1952a, b, c, 1954) and of isolated notopods (Anctil, 1981), as well as the effects of putative transmitters on luminescent responses (Anctil, 1981). These studies established that: (1) single and repetitive shocks applied to the ventral nerve cord or

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to isolated parapods can elicit luminescence, (2) section of the nerve cord alters the normal propagation of the luminescent response, (3) the responses are subject to facilitation, summation, and fatigue, and (4) acetylcholine has excitatory and gamma aminobutyric acid (GABA) inhibitory effects on isolated parapods. These observations led to the conclusion that the luminescent epithelium is controlled by the nervous system and that antagonistic cholinergic and GABAergic pathways mediate this control at the periphery. Studies on the histology (Nicol, 1952a) and ultrastructure (Anctil, 1979) of the luminescent epithelia of *Chaetopterus* disclosed a subepidermal nerve plexus with some of its neurites making junctional contacts with musculo-epithelial cells. The latter are apparently responsible for the extrusion of the luminescent mucus through their squeezing action on the mucous cells (Anctil, 1979).

While the nervous system appears to be involved in the regulation of light emission in *Chaetopterus,* there is almost no information available on how neural coordination of luminescence is achieved. This information could provide clues as to possible uses the animal could make of its luminescence. The principal aim of this paper was to build on Nicol's (1952b, c) observations and elucidate the relationship between nerve cord activity and light emission in *Chaetopterus* using photometric and electrophysiological recording techniques. We also report an as yet undescribed photogenic gland whose triggering mechanism differs greatly from that of the regular luminescent epithelium.

Materials and Methods

Maintenance and handling of specimens

Natural parchment tubes containing specimens of *Chaetopterus variopedatus* were obtained from Pacific Bio-Marine Laboratories (Venice, California). They were maintained in an aquarium system containing aerated, filtered, and recirculated artificial sea water (Instant Ocean). Temperature (14–16°C), salinity (35.5 ppm), and pH (8.4) were checked regularly. The worms were kept in darkness except for short periods of handling.

Due to the extremely delicate texture of the specimens and the ease with which luminescence can be evoked accidentally, great care was taken in the handling of the worms. Since chemical anesthesia inhibited light emission, specimens were immobilized in cold sea water (0°C). Each tube was then opened and the resident worm immediately placed in a petri dish containing sea water precooled to 0°C. The specimen was pinned tightly through the distal ends of the parapods on the Sylgard-coated bottom of a petri dish prior either to photometric recordings or exposing the nerve cord for electrophysiological recordings. All experiments were carried out at 20–21°C.

Photometric and electrophysiological recordings

In addition to visual records of luminescence by the dark-adapted (20–25 min) observer, photometric recordings of light emission following mechanical and electrical stimulation also were made. For this purpose, a fiber light guide was connected to a photomultiplier tube (EMI 9601B) which was activated by a high voltage power supply (EMI Gencom 3000R). The signal was received by a Grass 7P amplifier and recorded on a Grass 79D polygraph. A manual shutter was operated between the light guide and the photomultiplier to permit distinguishing dark noise from spontaneous activities of the preparation.

For electrophysiology, extracellular recordings were made using glass suction electrodes with a diameter of 20–100 μm. A chlorided silver wire was introduced coaxially

into the electrode tip which was polished over an alcohol burner. To avoid interference arising from the large quantities of mucus produced by the worm, good electrode contact was achieved by connecting the latter to a suction chamber; suction was controlled by connecting the chamber to a three-way valve and a 1-ml disposable syringe. The suction chamber was placed on a micromanipulator for precise positioning of the electrode tip viewed through a dissecting microscope.

Signals were fed to a Grass P511 preamplifier whose bandwidth was adjusted between 1 and 30 Hz. The amplified signals were received by a Tektronix 5113 oscilloscope for instantaneous visualization and by a Grass 79D polygraph for permanent records.

Stimulation

Electrical stimulation was applied through fine teflon-coated platinum electrodes which were connected to a Grass stimulation isolation unit and fed with square pulses from a Grass S44 stimulator.

Mechanical stimulation of limited epithelial areas of the parapods was delivered through a glass rod with a smooth, rounded end. The rod was clamped to a galvanometer (Grass 70SC D'Arsenval oscillograph). The application of D.C. current (10–50V) from a Grass stimulator to the galvanometer allowed control over the amplitude and duration of the displacement of the probe. The force delivered by this experimental set-up ranged between 0.015 (10V) and 0.038 dyne (50V) as estimated with a Grass FT.03 force transducer.

RESULTS

Electrical stimulation

Suprathreshold stimulation of any serial parapod by a single pulse led to a local luminescent response. As stimulus intensity was increased, luminescence spread to neighboring parapods, both anteriorly and posteriorly. First, ipsilateral parapods were recruited followed by contralateral parapods at higher stimulus intensities (Fig. 1).

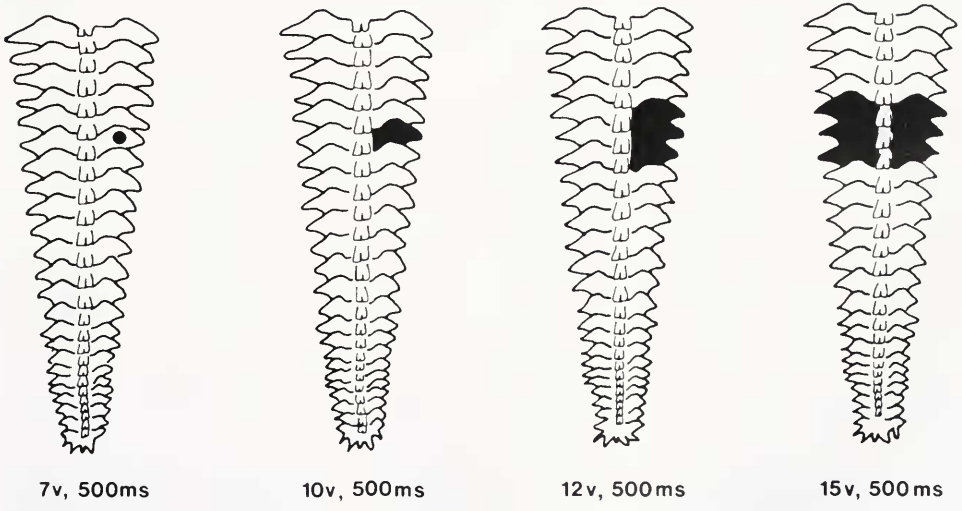

FIGURE 1. Schematic representation of recruitment of luminescent zones in posterior parapods in response to a single pulse of 500 ms of increasing intensity, applied to one parapod (see dot). Black regions represent sites of light emission.

The apparent intensity of the light emission of a parapod diminished as a function of its distance from the site of stimulation. Only a few segments could be recruited in this fashion, and luminescence was not propagated further either anteriorly or posteriorly in the 6 specimens tested.

A mild stimulation with square pulses (7V, 500 ms) on the surface of either aliform notopods of the 12th segment induced a weak, local luminescent response. With successive increases of stimulus intensity, luminescence became brighter and spread to the contralateral notopod, the middle segments, and eventually to all segments of the anterior and posterior regions, except for the photogenic gland of the 10–12th segments which remained unresponsive (see below). Thus, in contrast to other segments, the aliform notopods appeared to be preferentially accessible to the conduction pathway for luminescence propagation. The suspected substrate for this pathway, the ventral nerve cord, was then examined.

Stimulation of the nerve cord between segments 3 and 17 confirmed Nicol's (1952b) finding that the luminescent response spread in a discontinuous sequence, luminescence from posterior segments being recruited before that of middle or even anterior segments. Nevertheless, nerve cord stimulation consistently induced bilateral responses with a more rapid and widespread propagation of luminescence than notopodial stimulation. Reproducible propagation of luminescence in a more continuous antero-posterior sequence was achieved by electrical stimulation of the cerebral ganglion (see Martin and Anctil, 1984 for neuroanatomy). The same sequence of propagation was elicited by increasing the intensity (Fig. 2) or frequency of stimulation. Stimulation of the nerve cord at the level of the aliform notopods with single pulses or trains of pulses (Fig. 3) led to a response of low amplitude in these notopods and at the margin of the first fan in the 14th segment; then luminescence intensified and spread to other middle segments, and to the segments of the posterior and anterior regions following successive increases of stimulus intensity (Fig. 3).

Propagation of luminescent responses was more readily achieved in the antero-posterior than reverse direction. A stimulating pulse of 50 V, applied on the nerve cord of the last posterior segment, was necessary to elicit a response in the adjacent

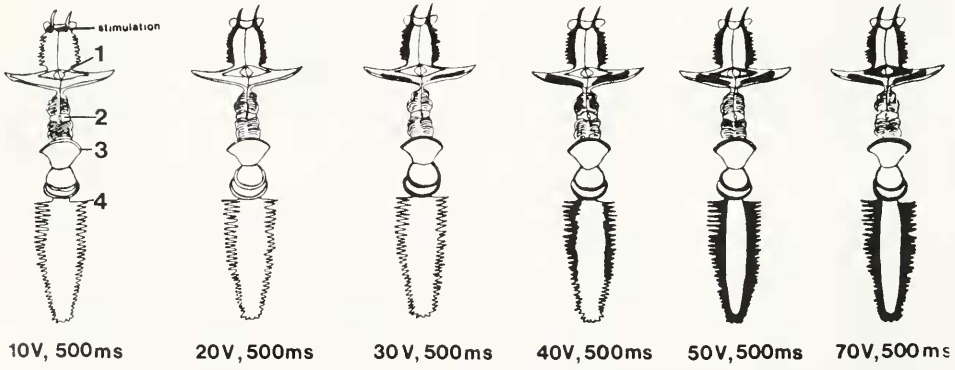

FIGURE 2. Schematic representation of recruitment of luminescent zones in response to a single stimulus pulse of increasing voltage, applied to the cerebral ganglion. Dot in extreme left specimen represents the site of stimulation and the black regions the sites of light emission. 1, Position of photogenic gland in 12th segment; 2, food cup in 13th segment; 3, first fan in 15th segment; 4, 18th segment where posterior segments begin. Note that photogenic gland in 12th segment failed to respond to a stimulus of 70V (extreme right). Data based on observations of six specimens.

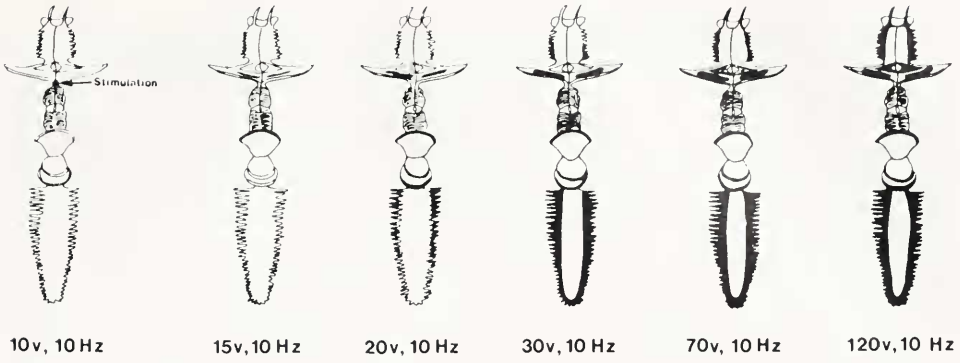

FIGURE 3. Schematic representation of recruitment of luminescent zones in response to pulse trains, lasting 2 s, of increasing voltage and applied to the nerve cord in the 12th segment. Symbols as in Figure 2. Data based on observations of six specimens.

segment. In contrast, a pulse of 10–20 V applied to more anterior segments was sufficient to elicit posterior spread in the same preparation.

Section of the nerve cord

The portion of the nerve cord associated with a single posterior segment was first isolated from the rest of the cord by double section. Electrical stimulation of this isolated portion elicited a light emission confined to both parapods of the associated segment in all specimens tested. Section of the nerve cord in the posterior region of the worm blocked the propagation of luminescence following electrical stimulation anteriorly (Fig. 4). Unilateral section of the widely separated left or right rami of the anterior nerve cord (see Martin and Anctil, 1984) had no effect on the propagation of bilateral luminescence, which still progressed posterior to the level of section. The conduction pathway for luminescence thus appeared to involve not only the two anterior nerve cords but also the numerous commissures interconnecting them.

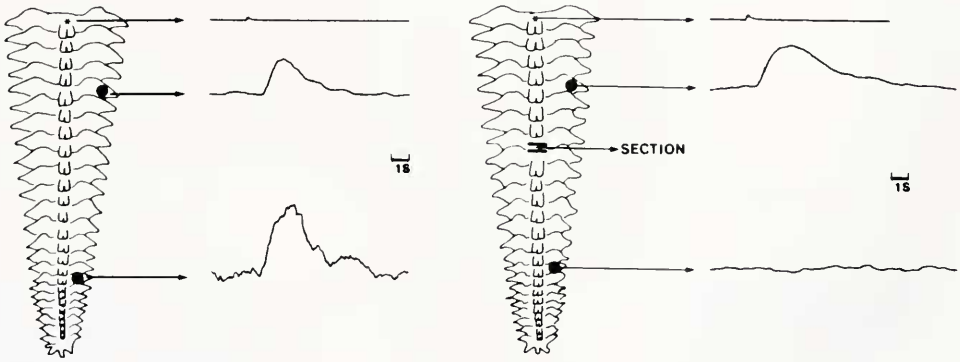

FIGURE 4. Effect of sectioning the nerve cord of the posterior region on propagation of luminescence. Left, intact nerve cord; right, after section of nerve cord. Upper trace, electrical stimulation (single pulse of 15 V, 500 ms); middle and lower traces, recordings of light emission. These observations were similarly obtained in all three specimens tested. Note failure of luminescence spread in the lower right recording.

Electrical activity in the nerve cord

Even in the non-luminescing worm the nerve cord continuously displayed arrhythmic signals, usually of very low amplitude. In contrast, light emission from any parapod was preceded by an increase in the electrical activity of the corresponding segmental ganglion in all 12 specimens investigated. This electrical activity was either spontaneous or electrically induced. Figure 5 illustrates two examples of simultaneous recordings of spontaneous impulse discharges and accompanying light emission.

These volleys varied in spike amplitude and frequency, and lasted between 0.5 and 2 s. The delay between initiation of the volley and onset of light emission ranged from 100 to 150 ms. Although the luminescent episodes varied in their rise and decay kinetics, no clear correlation between these and volley parameters could be made. Using two recording electrodes placed at varying distances from each other along the nerve cord, the conduction velocity of the volleys associated with luminescence was estimated to vary between 5 and 8 cm s^{-1}. The neural discharges associated with luminescence were conducted more readily in the antero-posterior than the reverse direction.

An additional volley often occurred near the onset or unfolding of the decaying phase of the luminescent response (Fig. 5a). Since the decaying phase reflects the chemical extinction of the extracellular luminescent reaction (Johnson, 1959) and/or the displacement of luminescent mucus away from the detection perimeter of the light guide, it is possible that this late discharge was associated with muscular activity resulting in mucus displacement.

Mechanical stimulation and the photogenic gland

Previous workers have stressed that the luminescent epithelium of *Chaetopterus* is highly sensitive to tactile stimulation or vibrations (see Nicol, 1952). This was confirmed by the difficulties in preparing the worms for experiments without inducing luminescence.

It was possible to produce controlled tactile stimulation on the epithelium using a galvanometer-controlled probe. Such a stimulus, applied to parapods, invariably induced a local luminescent response which failed to propagate to adjacent parapods despite the fact that it elicited a short impulse discharge in the segmental ganglion corresponding to the stimulated parapod (Fig. 6). This discharge, whose amplitude

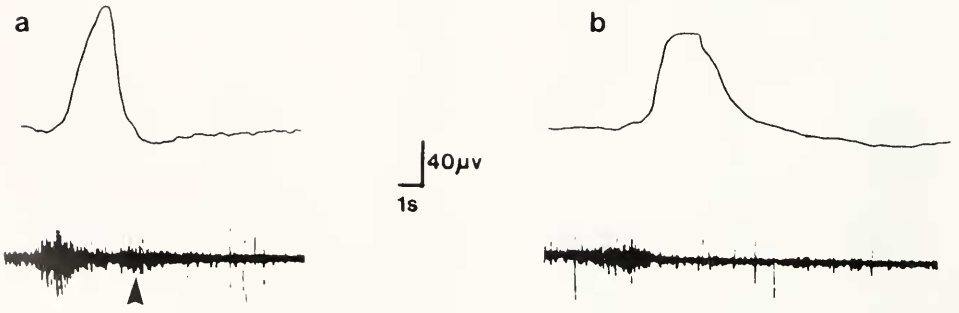

FIGURE 5. Recordings illustrating the relationship between spontaneous electrical activity in the nerve cord (lower trace) and spontaneous luminescent events (upper trace). In A the impulse discharge lasted 1.2 s and accompanying luminescence, 4 s; in B these parameters were of longer duration (2.2 s and 7.5 s, respectively). Note the after-discharge (arrowhead) in the nerve cord in A.

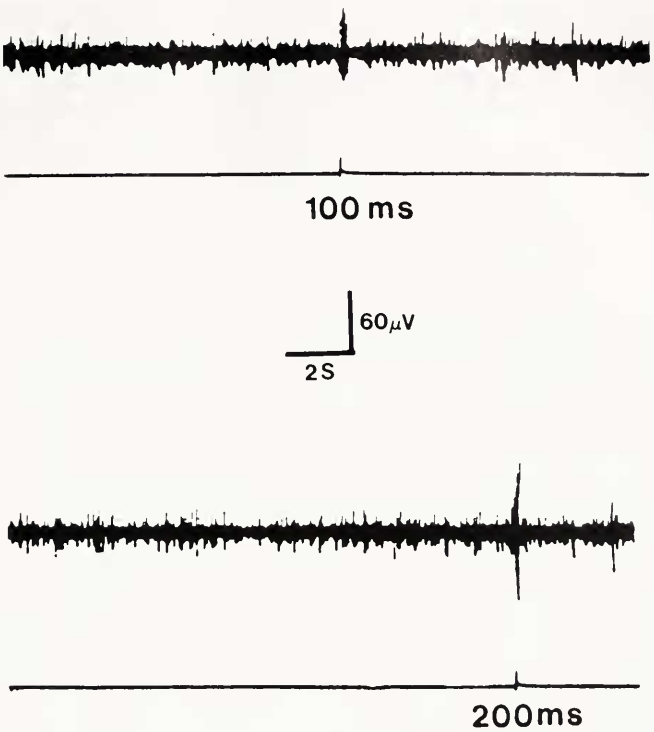

FIGURE 6. Recordings of electrical activity in the nerve cord (upper trace) in response to mechanical stimulation (.015 dyne) of the parapodial epidermis from the corresponding posterior segment (lower trace). Note the increment of impulse amplitude as a function of stimulus duration. Similar responses were obtained in all three specimens tested.

was dependent on stimulus strength or duration, was of much shorter duration than those associated with propagated luminescent responses (Fig. 5).

The photogenic gland appears as a swollen mass, 2–3 mm in diameter, and yellowish in live, sexually mature animals. It extends dorso-medially from the 10th segment to the intersection of the lateral and longitudinal ciliated grooves in the 12th segment (Fig. 2). Observations on serial histological sections from formalin-fixed, paraffin-embedded glands, stained with Mallory's triple stain, revealed that the gland consists of local enlargements of the glandular epithelium filled with mucous cells in a layer 700–800 μm deep at the expense of other cell types such as supportive and myoepithelial cells (Anctil, 1979). The photogenic gland, which was unresponsive to electrical stimulation either of the notopodial surface or of the nerve cord (Figs. 2, 3), consistently responded to direct mechanical handling by the release of a large quantity of brightly luminescent mucus in the water. The type of stimulation necessary to produce such a response (pinching or application of pressure on the gland) caused a rupture of the overlying epithelium of these glands, as visualized through a dissecting microscope. Thus epithelial rupture was an apparent prerequisite for expulsion of the mucus.

In order to assess the significance of mechanical disturbances in the context of tube-dwelling, eight tubes with their resident worm still inside were subjected to mechanical agitation in an aquarium. Following vigorous shaking of one extremity

of the tube with the fingers, a copious amount of luminescent mucus was almost instantaneously expelled through the agitated end in all trials (Fig. 7-1). The intense and bluish luminous cloud thus produced in the surrounding water was visible to the dark-adapted eye for 2–3 min. A quick examination of the inside of the tube revealed that this forceful release was immediately accompanied by a complete reversal of the worm's orientation in the tube (Fig. 7-2), the worm now positioned in a contracted state at the other end of the tube.

Discussion

This study conclusively establishes that except for the photogenic gland of segments 10–12 the nerve cord has an essential role in the excitation and propagation of the epithelial luminescent response of *Chaetopterus*. The cerebral ganglia, which are poorly developed in *Chaetopterus* (Martin and Anctil, 1984), may play a role in the coordination of luminescence propagation but are not necessary for the propagation itself. This is shown by the orderly spatial sequence of luminescence propagation following stimulation of a cerebral ganglion and the experiments on electrical stimulation of intact or sectioned nerve cord.

The luminescent responses to electrical stimulation of parapods and notopods are probably mediated by the nervous system for the following reasons: (1) luminescence spreads, albeit to a limited extent, as a function of the intensity of stimulation, (2) electrical stimulation of the aliform notopods, which are characterized by a much greater concentration of neurites than the parapods of the other segments (Anctil, 1979; Martin and Anctil, 1984), is the only means, short of direct nerve cord stimulation, by which to achieve through-conduction of luminescence excitation, and (3) a well-developed subepidermal nerve plexus is present throughout the body wall (Anctil, 1979; Martin and Anctil, 1984) and may act as the medium for decremental conduction of excitation at the periphery. It is unlikely, however, that intersegmental propagation of luminescence is mediated directly through the plexus since the latter does not appear to function as a true nerve net, at least in oligochaetes and polychaetes (Prosser, 1935, 1950; Mill, 1978). Furthermore, sectioning the nerve cord while leaving

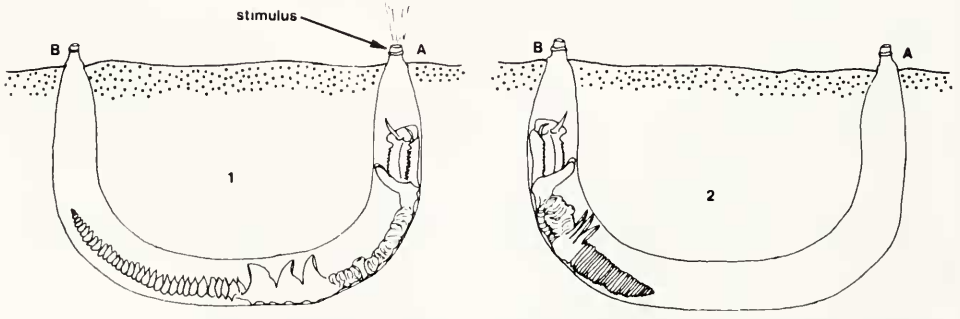

Figure 7. Schematic representation illustrating the relationship between the expulsion of luminescent mucus (1A) and the reversal of the worm's position at the other end of the tube (2B) in response to mechanical agitation of the tube. See text for further explanation.

the plexus intact is sufficient to block intersegmental propagation of stimulated luminescence.

Stimulation experiments on parapods suggest that the limited propagation of luminescence thus produced is dependent on a relatively small number of neurites recruited for conduction and on synaptic barriers that the nerve cord must overcome to transmit the excitation across the connectives and commissures linking the segments and the two cords, respectively. This is substantiated by the local luminescent response and accompanying nerve cord activity elicited by the mild mechanical stimulation of a small patch of parapodial epidermis. This presumably small sensory field would command a limited number of afferents to the nerve cord, probably insufficient to overcome the threshold level set by the synaptic layout of the local ganglionic neuropile.

In contrast, propagation of luminescence through the entire body is readily achieved by nerve cord stimulation. We have identified impulse discharges which travel along the ventral nerve cord and are associated with luminescent episodes, either spontaneous or electrically stimulated. These temporally correlated activities provide additional support for the necessary role of nerve cord function in activating the luminescent epithelium. The high spiking frequency as well as the small and variable amplitude of spikes in these volleys probably reflect the activity of multiple neurites of small caliber. This view is consistent with measurements of axon diameters of 2 μm or less in the nerve cord of *Chaetopterus* (Martin and Anctil, 1984).

Two features of the nerve cord-mediated luminescence conduction deserve mention: the conduction velocity and the polarization of impulse conduction. The slow speeds of conduction (5–8 cm s^{-1}) are within the range reported in the nerve cord of other annelids where giant fibers are not involved and multiple synaptic interactions between segments occur (Bullock and Horridge, 1965). No giant fiber has been detected in *Chaetopterus* (Nicol, 1948; Martin and Anctil, 1984), and the great majority of the nerve fibers in the nerve cord were found to be less than 1 μm in diameter (Martin and Anctil, 1984). Bullock and Horridge (1965) reviewed evidence of a "preference for posterior propagation" in the nerve cord of polychaetes which we substantiate in the case of *Chaetopterus*. The functional morphology on which this behavior is based is uncertain, although the presence of a large number of polarized and of relatively few non-polarized (symmetrical) synapses in segmental nerves (Anctil, 1979) as well as in the nerve cord (Martin and Anctil, 1984) could be a contributing factor.

Discrete mechanical stimuli readily induced luminescent responses in *Chaetopterus*. These local responses might be elicited through a reflex pathway involving sensory cells, such as the ciliated cells in the epidermis of *Chaetopterus* (Anctil, 1979), whose excitation would be transmitted to the subepidermal nerve plexus and finally to the myoepithelial cells mediating mucus release. The nerve cord activity recorded following stimulation of the same segment was probably induced by the subepidermal plexus. Therefore it is likely that large patches of sensory epidermis must be simultaneously activated to initiate propagated nerve cord impulses and consequent spread of luminescent responses such as is witnessed by vigorously handling the worm.

The refractoriness of the photogenic gland to electrical or mild mechanical stimulation, which has not been reported by previous investigators, is of particular interest in view of its large store of luminescent mucus and impressive luminescent display. What could be the biological significance of a structure that must be damaged in order to elicit a luminescent discharge? Brown and Rosen (1978) described a tube cleaning behavior in *Chaetopterus* involving a reversal of the orientation of its body inside the tube. This reversal is accomplished by raising and folding the anterior region of the body in the posterior direction, and this is accompanied by a torsion of the body. This is likely to cause a marked compression of the photogenic gland,

leading possibly to the rupture of its epithelium and the propulsion of a large amount of luminescent mucus. The change of position followed by vigorous movements of the fans of the mid-region of the body (Brown and Rosen, 1978) cause a reversal of the direction of the water flow in the tube and, consequently, the forceful expulsion of luminescent mucus as well as undesirable detritus material or intruders. The propulsive force is enhanced by the locomotory activity of the worm toward the opposite end of its tube which is in the same direction as that of the water pumping activity.

Thus, according to our hypothesis, the photogenic gland is not under direct nervous control but becomes involved directly as a result of mechanical stress produced by motor activities associated with the tube-cleaning behavior or mechanical agitation of the tube. Indeed, our observations show that vigorous mechanical agitation of the tube not only induces a forceful ejection of luminescent mucus, but also causes the reversal of position and locomotory activity previously reported by Brown and Rosen (1978). It is also possible that the luminescent mucus from the regular luminescent epithelium of the parapods is released following multiple disturbances comparable to those causing the ejection of mucus from the photogenic glands. It should be emphasized however that the amount of mucus the luminescent epithelium can mobilize in such a short time is insufficient to account for the impressive cloud of luminescence witnessed under these conditions.

These observations suggest a possible role for bioluminescence in *Chaetopterus*. The worm appears to react similarly to invasion of its tube by foreign materials and outside attacks on the tube. The reaction involves a defensive retreat of the worm to one end of the tube and the expulsion of a bright cloud of luminescent mucus at the opposite end. The dispersal of that cloud in the water would then confuse the potential attacker as to the exact location of its prey. There are still unresolved problems with this model, namely the relative contributions of the photogenic gland and neurally controlled luminescent epithelium to the expelled luminous cloud, and the role of the ventral nerve cord in the integration of the sensoro-motor activities associated with this behavior.

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